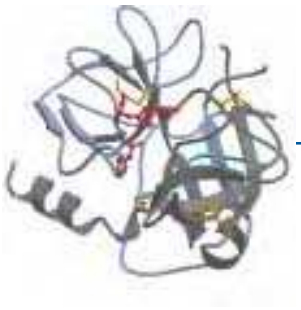


PART II

BIOENERGETICS AND METABOLISM

Metabolism is a highly coordinated cellular activity in which many multienzyme systems (metabolic pathways) cooperate to (1) obtain chemical energy by capturing solar energy or degrading energy-rich nutrients from the environment; (2) convert nutrient molecules into the cell's own characteristic molecules, including precursors of macromolecules; (3) polymerize monomeric precursors into macromolecules: proteins, nucleic acids, and polysaccharides; and (4) synthesize and degrade biomolecules required for specialized cellular functions, such as membrane lipids, intracellular messengers, and pigments.

Although metabolism embraces hundreds of different enzyme-catalyzed reactions, our major concern in Part II is the central metabolic pathways, which are few in number and remarkably similar in all forms of life. Living organisms can be divided into two large groups according to the chemical form in which they obtain carbon from the environment. **Autotrophs** (such as photosynthetic bacteria and vascular plants) can use carbon dioxide from the atmosphere as their sole source of carbon, from which they construct all their carbon-containing biomolecules (see Fig. 1–5). Some autotrophic organisms, such as cyanobacteria, can also use atmospheric nitrogen to generate all their nitrogenous components. **Heterotrophs** cannot use atmospheric carbon dioxide and must obtain carbon from their environment in the form of relatively complex organic molecules such as glucose. Multicellular animals and most microorganisms are heterotrophic. Autotrophic cells and organisms are relatively self-sufficient, whereas heterotrophic cells and organisms, with their requirements for carbon in more complex forms, must subsist on the products of other organisms.

Many autotrophic organisms are photosynthetic and obtain their energy from sunlight, whereas heterotrophic organisms obtain their energy from the degradation of organic nutrients produced by autotrophs. In our biosphere, autotrophs and heterotrophs live together in a vast, interdependent cycle in which autotrophic organisms use atmospheric carbon dioxide to build their organic biomolecules, some of them generating oxygen from water in the process. Heterotrophs in turn use the organic products of autotrophs as nutrients and return carbon dioxide to the atmosphere. Some of the oxidation reactions that produce carbon dioxide also consume oxygen, converting it to water. Thus carbon, oxygen, and water are constantly cycled between the heterotrophic and autotrophic worlds, with

solar energy as the driving force for this global process (Fig. 1).

All living organisms also require a source of nitrogen, which is necessary for the synthesis of amino acids, nucleotides, and other compounds. Plants can generally use either ammonia or nitrate as their sole source of nitrogen, but vertebrates must obtain nitrogen in the form of amino acids or other organic compounds. Only a few organisms—the cyanobacteria and many species of soil bacteria that live symbiotically on the roots of some plants—are capable of converting ("fixing") atmospheric nitrogen (N_2) into ammonia. Other bacteria (the nitrifying bacteria) oxidize ammonia to nitrites and nitrates; yet others convert nitrate to N_2. Thus, in addition to the global carbon and oxygen cycle, a nitrogen cycle operates in the biosphere, turning over huge amounts of nitrogen (Fig. 2). The cycling of carbon, oxygen, and nitrogen, which ultimately involves all species, depends on a proper balance between the activities of the producers (autotrophs) and consumers (heterotrophs) in our biosphere.

These cycles of matter are driven by an enormous flow of energy into and through the biosphere, beginning with the capture of solar energy by photosynthetic organisms and use of this energy to generate energy-rich carbohydrates and other organic nutrients; these nutrients are then used as energy sources by heterotrophic organisms. In metabolic processes, and in all energy transformations, there is a loss of useful energy (free energy) and an inevitable increase in the amount of unusable energy (heat and entropy). In contrast to the cycling of matter, therefore, energy flows one way through the biosphere; organisms cannot regenerate useful energy from energy dissipated as heat and entropy. Carbon, oxygen, and nitrogen recycle continuously, but energy is constantly transformed into unusable forms such as heat.

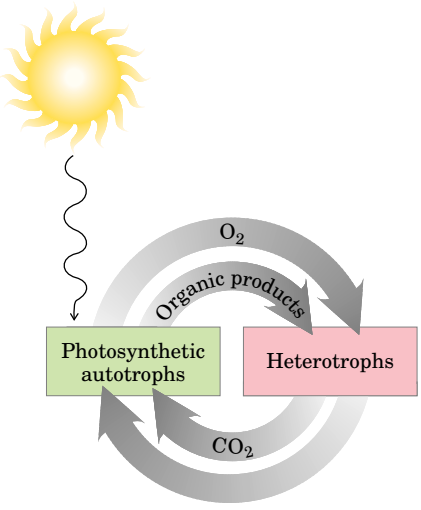


FIGURE 1 **Cycling of carbon dioxide and oxygen between the autotrophic (photosynthetic) and heterotrophic domains in the biosphere.** The flow of mass through this cycle is enormous; about 4×10^{11} metric tons of carbon are turned over in the biosphere annually.

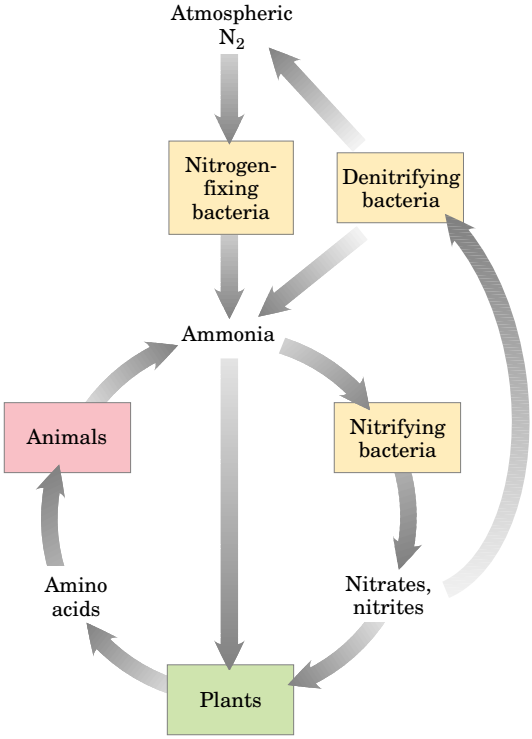


FIGURE 2 **Cycling of nitrogen in the biosphere.** Gaseous nitrogen (N_2) makes up 80% of the earth's atmosphere.

Metabolism, the sum of all the chemical transformations taking place in a cell or organism, occurs through a series of enzyme-catalyzed reactions that constitute **metabolic pathways.** Each of the consecutive steps in a metabolic pathway brings about a specific, small chemical change, usually the removal, transfer, or addition of a particular atom or functional group. The precursor is converted into a product through a series of metabolic intermediates called **metabolites.** The term **intermediary metabolism** is often applied to the combined activities of all the metabolic pathways that interconvert precursors, metabolites, and products of low molecular weight (generally, $M_r < 1{,}000$).

Catabolism is the degradative phase of metabolism in which organic nutrient molecules (carbohydrates, fats, and proteins) are converted into smaller, simpler end products (such as lactic acid, CO_2, NH_3). Catabolic pathways release energy, some of which is conserved in the formation of ATP and reduced electron carriers (NADH, NADPH, and $FADH_2$); the rest is lost as heat. In **anabolism,** also called biosynthesis, small, simple precursors are built up into larger and more complex

molecules, including lipids, polysaccharides, proteins, and nucleic acids. Anabolic reactions require an input of energy, generally in the form of the phosphoryl group transfer potential of ATP and the reducing power of NADH, NADPH, and $FADH_2$ (Fig. 3).

Some metabolic pathways are linear, and some are branched, yielding multiple useful end products from a single precursor or converting several starting materials into a single product. In general, catabolic pathways are convergent and anabolic pathways divergent (Fig. 4). Some pathways are cyclic: one starting component of the pathway is regenerated in a series of reactions that converts another starting component into a product. We shall see examples of each type of pathway in the following chapters.

Most cells have the enzymes to carry out both the degradation and the synthesis of the important categories of biomolecules—fatty acids, for example. The simultaneous synthesis and degradation of fatty acids would be wasteful, however, and this is prevented by reciprocally regulating the anabolic and catabolic reaction sequences: when one sequence is active, the other is suppressed. Such regulation could not occur if anabolic and catabolic pathways were catalyzed by exactly the same set of enzymes, operating in one direction for anabolism, the opposite direction for catabolism: inhibition of an enzyme involved in catabolism would also inhibit the reaction sequence in the anabolic direction. Catabolic and anabolic pathways that connect the same two end points (glucose $\rightarrow\rightarrow$ pyruvate and pyruvate $\rightarrow\rightarrow$ glucose, for example) may employ many of the same enzymes, but invariably at least one of the steps is catalyzed by different enzymes in the catabolic and anabolic directions, and these enzymes are the sites of separate regulation. Moreover, for both anabolic and catabolic pathways to be essentially irreversible, the reactions unique to each direction must include at least one that is thermodynamically very favorable—in other words, a reaction for which the reverse reaction is very unfavorable. As a further contribution to the separate regulation of catabolic and anabolic reaction sequences, paired catabolic and anabolic pathways commonly take place in different cellular compartments: for example, fatty acid catabolism in mitochondria, fatty acid synthesis in the cytosol. The concentrations of intermediates, enzymes, and regulators can be maintained at different levels in these different compartments. Because metabolic pathways are subject to kinetic control by substrate concentration, separate pools of anabolic and catabolic intermediates also contribute to the control of metabolic rates. Devices that separate anabolic and catabolic processes will be of particular interest in our discussions of metabolism.

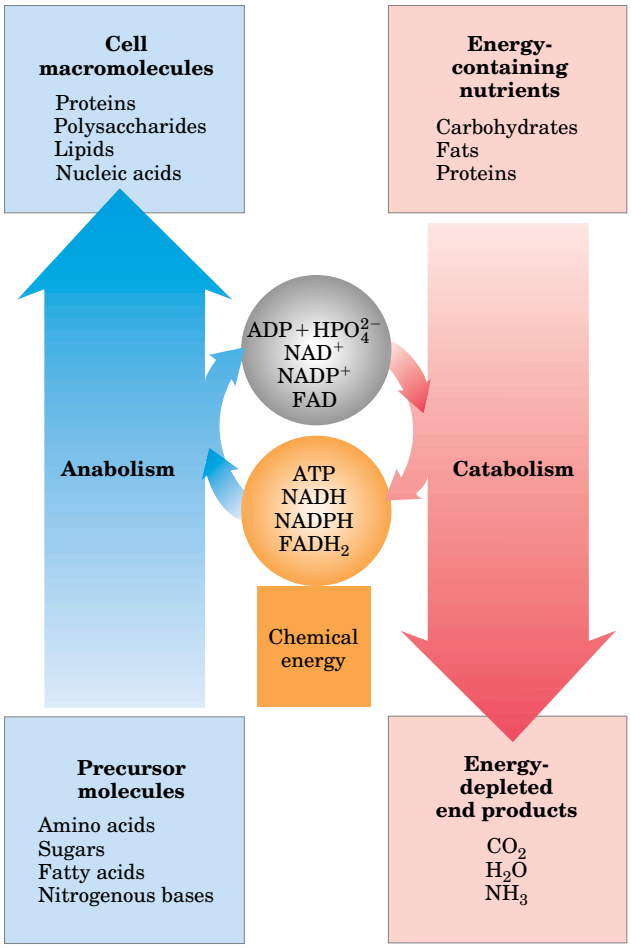


FIGURE 3 **Energy relationships between catabolic and anabolic pathways.** Catabolic pathways deliver chemical energy in the form of ATP, NADH, NADPH, and $FADH_2$. These energy carriers are used in anabolic pathways to convert small precursor molecules into cell macromolecules.

Metabolic pathways are regulated at several levels, from within the cell and from outside. The most immediate regulation is by the availability of substrate; when the intracellular concentration of an enzyme's substrate is near or below K_m (as is commonly the case), the rate of the reaction depends strongly upon substrate concentration (see Fig. 6–11). A second type of rapid control from within is allosteric regulation (p. 225) by a metabolic intermediate or coenzyme—an amino acid or ATP, for example—that signals the cell's internal metabolic state. When the cell contains an amount of, say, aspartate sufficient for its immediate needs, or when the cellular level of ATP indicates that further fuel consumption is unnecessary at the moment, these signals allosterically inhibit the activity of one or more enzymes in the relevant pathway. In multicellular organisms the metabolic activities of different tissues are regulated and integrated by growth factors and hormones that act from outside the cell. In some cases this regulation occurs virtually instantaneously (sometimes in less than a millisecond) through changes in the levels of intracellular

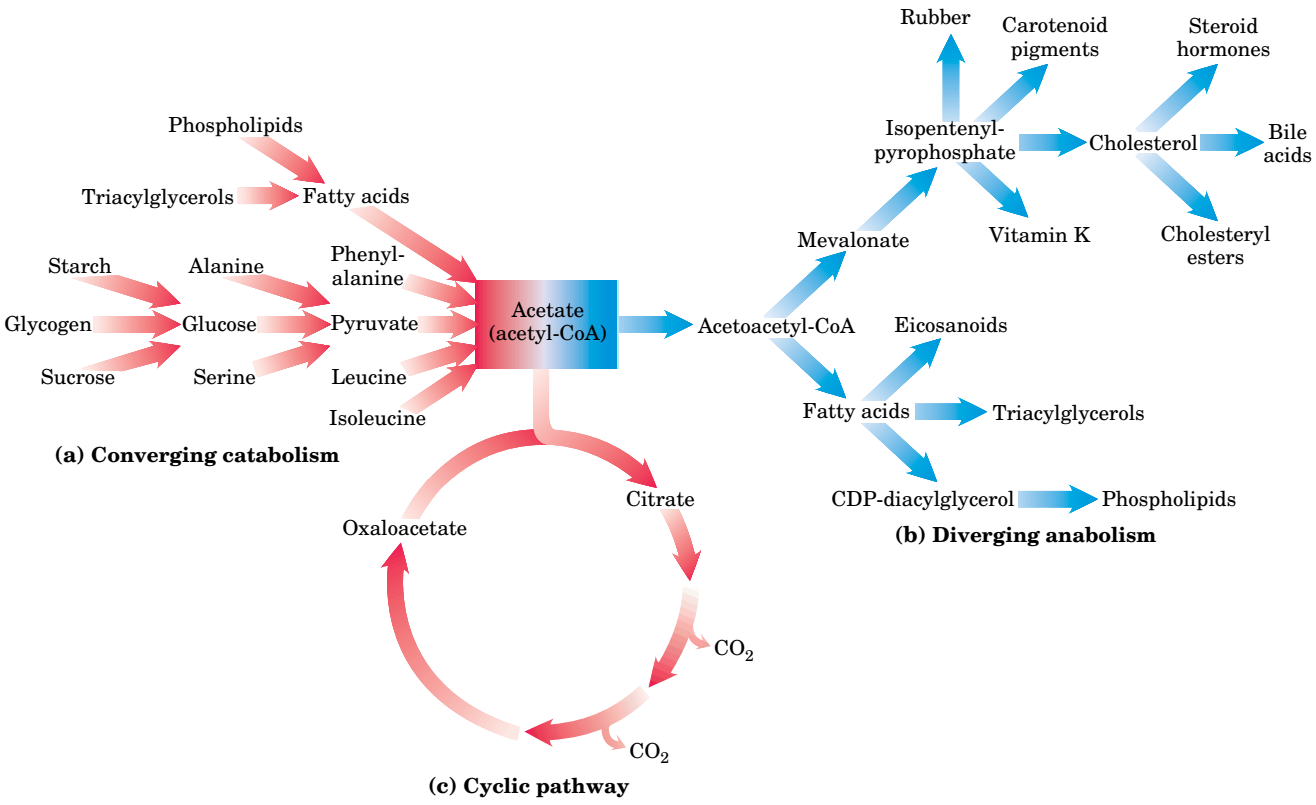


FIGURE 4 **Three types of nonlinear metabolic pathways. (a)** Converging, catabolic; **(b)** diverging, anabolic; and **(c)** cyclic, in which one of the starting materials (oxaloacetate in this case) is regenerated and reenters the pathway. Acetate, a key metabolic intermediate, is the breakdown product of a variety of fuels **(a),** serves as the precursor for an array of products **(b),** and is consumed in the catabolic pathway known as the citric acid cycle **(c).**

messengers that modify the activity of existing enzyme molecules by allosteric mechanisms or by covalent modification such as phosphorylation. In other cases, the extracellular signal changes the cellular concentration of an enzyme by altering the rate of its synthesis or degradation, so the effect is seen only after minutes or hours.

The number of metabolic transformations taking place in a typical cell can seem overwhelming to a beginning student. Most cells have the capacity to carry out thousands of specific, enzyme-catalyzed reactions: for example, transformation of a simple nutrient such as glucose into amino acids, nucleotides, or lipids; extraction of energy from fuels by oxidation; or polymerization of monomeric subunits into macromolecules. Fortunately for the student of biochemistry, there are patterns within this multitude of reactions; you do not need to learn all these reactions to comprehend the molecular logic of biochemistry. Most of the reactions in living cells fall into one of five general categories: (1) oxidation-reductions; (2) reactions that make or break carbon–carbon bonds; (3) internal rearrangements, isomerizations, and eliminations; (4) group transfers; and (5) free radical reactions. Reactions within each general category usually proceed by a limited set of mechanisms and often employ characteristic cofactors.

Before reviewing the five main reaction classes of biochemistry, let's consider two basic chemical principles. First, a covalent bond consists of a shared pair of electrons, and the bond can be broken in two general ways (Fig. 5). In homolytic cleavage, each atom leaves the bond as a radical, carrying one of the two electrons (now unpaired) that held the bonded atoms together. In the more common, heterolytic cleavage, one atom retains both bonding electrons. The species generated when C—C and C—H bonds are cleaved are illustrated in Figure 5. Carbanions, carbocations, and hydride ions are highly unstable; this instability shapes the chemistry of these ions, as described further below.

The second chemical principle of interest here is that many biochemical reactions involve interactions between nucleophiles (functional groups rich in electrons and capable of donating them) and electrophiles (electron-deficient functional groups that seek electrons). Nucleophiles combine with, and give up electrons to, electrophiles. Common nucleophiles and electrophiles are listed in Figure 6–21. Note that a carbon atom can act as either a nucleophile or an electrophile, depending on which bonds and functional groups surround it.

We now consider the five main reaction classes you will encounter in upcoming chapters.

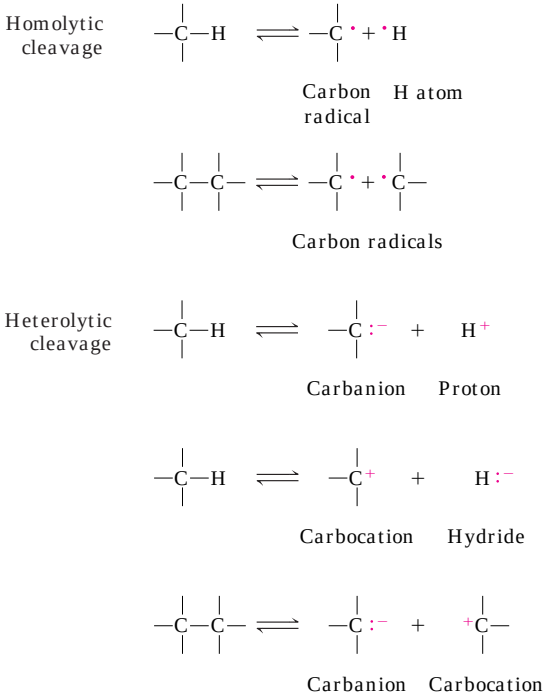


FIGURE 5 **Two mechanisms for cleavage of a C—C or C—H bond.** In homolytic cleavages, each atom keeps one of the bonding electrons, resulting in the formation of carbon radicals (carbons having unpaired electrons) or uncharged hydrogen atoms. In heterolytic cleavages, one of the atoms retains both bonding electrons. This can result in the formation of carbanions, carbocations, protons, or hydride ions.

1. Oxidation-reduction reactions Carbon atoms encountered in biochemistry can exist in five oxidation states, depending on the elements with which carbon shares electrons (Fig. 6). In many biological oxidations, a compound loses two electrons and two hydrogen ions (that is, two hydrogen atoms); these reactions are commonly called dehydrogenations and the enzymes that catalyze them are called dehydrogenases (Fig. 7). In some, but not all, biological oxidations, a carbon atom becomes covalently bonded to an oxygen atom. The enzymes that catalyze these oxidations are generally called oxidases or, if the oxygen atom is derived directly from molecular oxygen (O_2), oxygenases.

Every oxidation must be accompanied by a reduction, in which an electron acceptor acquires the electrons removed by oxidation. Oxidation reactions generally release energy (think of camp fires: the compounds in wood are oxidized by oxygen molecules in the air). Most living cells obtain the energy needed for cellular work by oxidizing metabolic fuels such as carbohydrates or fat; photosynthetic organisms can also trap and use the energy of sunlight. The catabolic (energy-yielding) pathways described in Chapters 14 through 19 are oxidative reaction sequences that result in the transfer of electrons from fuel molecules, through a series of electron carriers, to oxygen. The high affinity of O_2 for electrons makes the overall electron-transfer process highly exergonic, providing the energy that drives ATP synthesis—the central goal of catabolism.

2. Reactions that make or break carbon–carbon bonds Heterolytic cleavage of a C—C bond yields a carbanion and a carbocation (Fig. 5). Conversely, the formation of a C—C bond involves the combination of a nucleophilic carbanion and an electrophilic carbocation. Groups with electronegative atoms play key roles in these reactions. Carbonyl groups are particularly important in the chemical transformations of metabolic pathways. As noted above, the carbon of a carbonyl group has a partial positive charge due to the electron-withdrawing nature of the adjacent bonded oxygen, and thus is an electrophilic carbon. The presence of a carbonyl group can also facilitate the formation of a carbanion on an adjoining carbon, because the carbonyl group can delocalize electrons through resonance (Fig. 8a, b). The importance of a carbonyl group is evident in three major classes of reactions in which C—C bonds are formed or broken (Fig 8c): aldol condensations (such as the aldolase reaction; see Fig. 14–5), Claisen condensations (as in the citrate synthase reaction; see Fig. 16–9), and

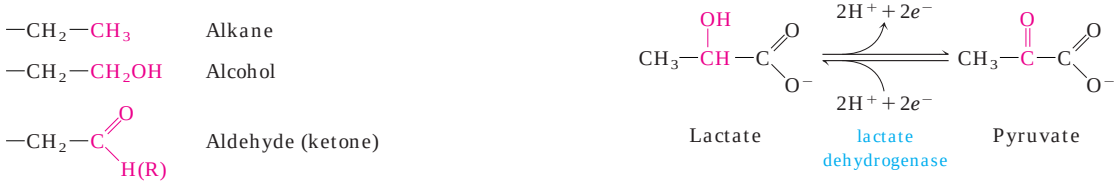


FIGURE 6 **The oxidation states of carbon in biomolecules.** Each compound is formed by oxidation of the red carbon in the compound listed above it. Carbon dioxide is the most highly oxidized form of carbon found in living systems.

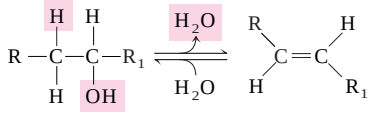
FIGURE 7 **An oxidation-reduction reaction.** Shown here is the oxidation of lactate to pyruvate. In this dehydrogenation, two electrons and two hydrogen ions (the equivalent of two hydrogen atoms) are removed from C-2 of lactate, an alcohol, to form pyruvate, a ketone. In cells the reaction is catalyzed by lactate dehydrogenase and the electrons are transferred to a cofactor called nicotinamide adenine dinucleotide. This reaction is fully reversible; pyruvate can be reduced by electrons from the cofactor. In Chapter 13 we discuss the factors that determine the direction of a reaction.

decarboxylations (as in the acetoacetate decarboxylase reaction; see Fig. 17–18). Entire metabolic pathways are organized around the introduction of a carbonyl group in a particular location so that a nearby carbon–carbon bond can be formed or cleaved. In some reactions, this role is played by an imine group or a specialized cofactor such as pyridoxal phosphate, rather than by a carbonyl group.

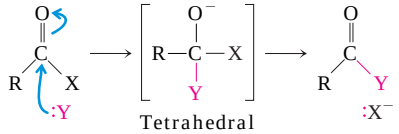
3. Internal rearrangements, isomerizations, and eliminations Another common type of cellular reaction is an intramolecular rearrangement, in which redistribution of electrons results in isomerization, transposition of double bonds, or cis-trans rearrangements of double bonds. An example of isomerization is the formation of fructose 6-phosphate from glucose 6-phosphate during sugar metabolism (Fig 9a; this reaction is discussed in detail in Chapter 14). Carbon-1 is reduced (from aldehyde to alcohol) and C-2 is oxidized (from alcohol to ketone). Figure 9b shows the details of the electron movements that result in isomerization.

A simple transposition of a C═C bond occurs during metabolism of the common fatty acid oleic acid (see Fig. 17–9), and you will encounter some spectacular examples of double-bond repositioning in the synthesis of cholesterol (see Fig. 21–35).

Elimination of water introduces a C═C bond between two carbons that previously were saturated (as in the enolase reaction; see Fig. 6–23). Similar reactions can result in the elimination of alcohols and amines.



4. Group transfer reactions The transfer of acyl, glycosyl, and phosphoryl groups from one nucleophile to another is common in living cells. Acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an acyl group to form a tetrahedral intermediate.



The chymotrypsin reaction is one example of acyl group transfer (see Fig. 6–21). Glycosyl group transfers involve nucleophilic substitution at C-1 of a sugar ring, which is the central atom of an acetal. In principle, the substitution could proceed by an S_N1 or S_N2 path, as described for the enzyme lysozyme (see Fig. 6–25).

Phosphoryl group transfers play a special role in metabolic pathways. A general theme in metabolism is the attachment of a good leaving group to a metabolic intermediate to "activate" the intermediate for subsequent reaction. Among the better leaving groups in nucleophilic substitution reactions are inorganic orthophosphate (the ionized form of H_3PO_4 at neutral pH, a mixture of $H_2PO_4^-$ and HPO_4^{2-}, commonly abbreviated P_i) and inorganic pyrophosphate ($P_2O_7^{4-}$, abbreviated PP_i); esters and anhydrides of phosphoric acid are effectively activated for reaction. Nucleophilic substitution is made more favorable by the attachment of a phosphoryl group to an otherwise poor leaving group such as —OH. Nucleophilic substitutions in which the

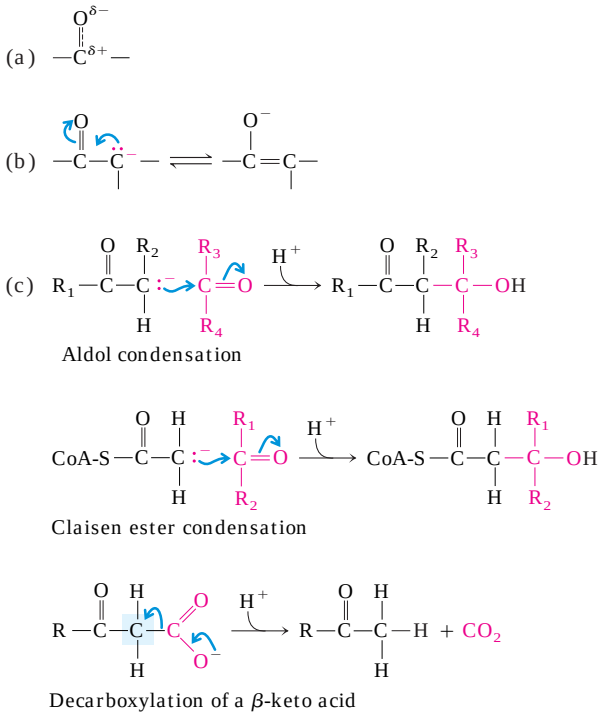


FIGURE 8 Carbon–carbon bond formation reactions. (a) The carbon atom of a carbonyl group is an electrophile by virtue of the electron-withdrawing capacity of the electronegative oxygen atom, which results in a resonance hybrid structure in which the carbon has a partial positive charge. **(b)** Within a molecule, delocalization of electrons into a carbonyl group facilitates the transient formation of a carbanion on an adjacent carbon. **(c)** Some of the major reactions involved in the formation and breakage of C—C bonds in biological systems. For both the aldol condensation and the Claisen condensation, a carbanion serves as nucleophile and the carbon of a carbonyl group serves as electrophile. The carbanion is stabilized in each case by another carbonyl at the carbon adjoining the carbanion carbon. In the decarboxylation reaction, a carbanion is formed on the carbon shaded blue as the CO_2 leaves. The reaction would not occur at an appreciable rate but for the stabilizing effect of the carbonyl adjacent to the carbanion carbon. Wherever a carbanion is shown, a stabilizing resonance with the adjacent carbonyl, as shown in **(a),** is assumed. The formation of the carbanion is highly disfavored unless the stabilizing carbonyl group, or a group of similar function such as an imine, is present.

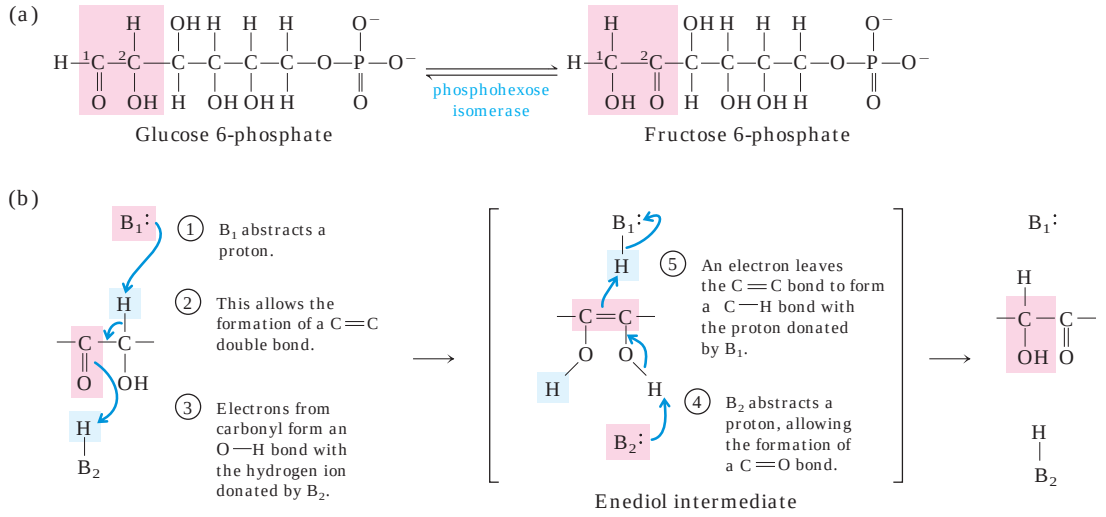


FIGURE 9 **Isomerization and elimination reactions. (a)** The conversion of glucose 6-phosphate to fructose 6-phosphate, a reaction of sugar metabolism catalyzed by phosphohexose isomerase. **(b)** This reaction proceeds through an enediol intermediate. The curved blue arrows represent the movement of bonding electrons from nucleophile (pink) to electrophile (blue). B_1 and B_2 are basic groups on the enzyme; they are capable of donating and accepting hydrogen ions (protons) as the reaction progresses.

phosphoryl group ($—PO_3^{2-}$) serves as a leaving group occur in hundreds of metabolic reactions.

Phosphorus can form five covalent bonds. The conventional representation of P_i (Fig. 10a), with three P—O bonds and one P=O bond, is not an accurate picture. In P_i, four equivalent phosphorus–oxygen bonds share some double-bond character, and the anion has a tetrahedral structure (Fig. 10b). As oxygen is more electronegative than phosphorus, the sharing of electrons is unequal: the central phosphorus bears a partial positive charge and can therefore act as an electrophile. In a very large number of metabolic reactions, a phosphoryl group ($—PO_3^{2-}$) is transferred from ATP to an alcohol (forming a phosphate ester) (Fig. 10c) or to a carboxylic acid (forming a mixed anhydride). When a nucleophile attacks the electrophilic phosphorus atom in ATP, a relatively stable pentacovalent structure is formed as a reaction intermediate (Fig. 10d). With departure of the leaving group (ADP), the transfer of a phosphoryl group is complete. The large family of enzymes that catalyze

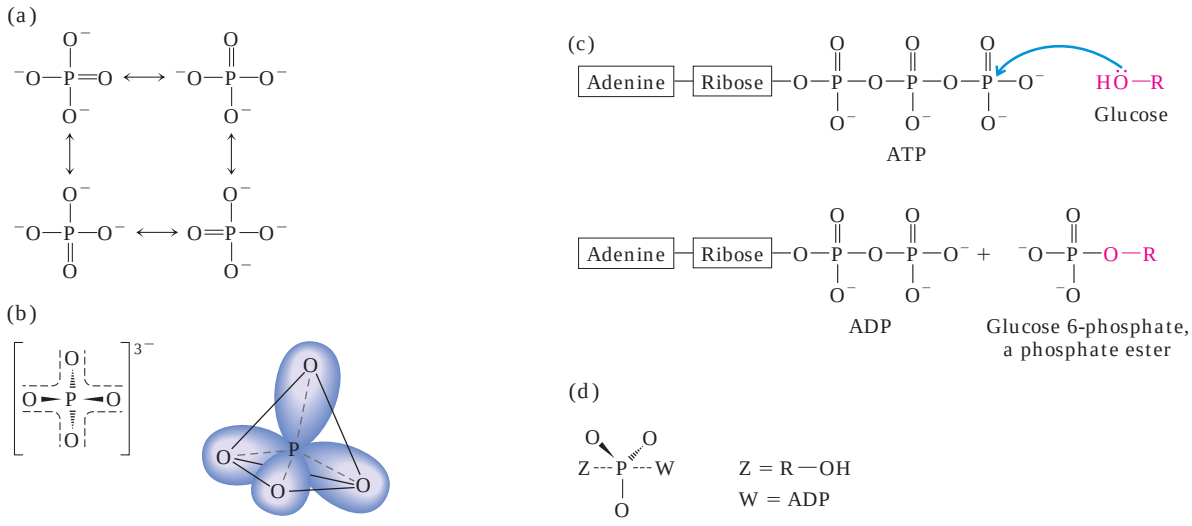


FIGURE 10 **Alternative ways of showing the structure of inorganic orthophosphate. (a)** In one (inadequate) representation, three oxygens are single-bonded to phosphorus, and the fourth is double-bonded, allowing the four different resonance structures shown. **(b)** The four resonance structures can be represented more accurately by showing all four phosphorus–oxygen bonds with some double-bond character; the hybrid orbitals so represented are arranged in a tetrahedron with P at its center. **(c)** When a nucleophile Z (in this case, the —OH on C-6 of glucose) attacks ATP, it displaces ADP (W). In this S_N2 reaction, a pentacovalent intermediate **(d)** forms transiently.

phosphoryl group transfers with ATP as donor are called kinases (Greek *kinein*, "to move"). Hexokinase, for example, "moves" a phosphoryl group from ATP to glucose.

Phosphoryl groups are not the only activators of this type. Thioalcohols (thiols), in which the oxygen atom of an alcohol is replaced with a sulfur atom, are also good leaving groups. Thiols activate carboxylic acids by forming thioesters (thiol esters) with them. We will discuss a number of cases, including the reactions catalyzed by the fatty acyl transferases in lipid synthesis (see Fig. 21–2), in which nucleophilic substitution at the carbonyl carbon of a thioester results in transfer of the acyl group to another moiety.

5. *Free radical reactions* Once thought to be rare, the homolytic cleavage of covalent bonds to generate free radicals has now been found in a range of biochemical processes. Some examples are the reactions of methylmalonyl-CoA mutase (see Box 17–2), ribonucleotide reductase (see Fig. 22–41), and DNA photolyase (see Fig. 25–25).

We begin Part II with a discussion of the basic energetic principles that govern all metabolism (Chapter 13). We then consider the major catabolic pathways by which cells obtain energy from the oxidation of various fuels (Chapters 14 through 19). Chapter 19 is the pivotal point of our discussion of metabolism; it concerns chemiosmotic energy coupling, a universal mechanism in which a transmembrane electrochemical potential, produced either by substrate oxidation or by light absorption, drives the synthesis of ATP.

Chapters 20 through 22 describe the major anabolic pathways by which cells use the energy in ATP to produce carbohydrates, lipids, amino acids, and nucleotides from simpler precursors. In Chapter 23 we step back from our detailed look at the metabolic pathways—as they occur in all organisms, from *Escherichia coli* to humans—and consider how they are regulated and integrated in mammals by hormonal mechanisms.

As we undertake our study of intermediary metabolism, a final word. Keep in mind that the myriad reactions described in these pages take place in, and play crucial roles in, living organisms. As you encounter each reaction and each pathway ask, What does this chemical transformation do for the organism? How does this pathway interconnect with the other pathways operating simultaneously in the same cell to produce the energy and products required for cell maintenance and growth? How do the multilayered regulatory mechanisms cooperate to balance metabolic and energy inputs and outputs, achieving the dynamic steady state of life? Studied with this perspective, metabolism provides fascinating and revealing insights into life, with countless applications in medicine, agriculture, and biotechnology.